American Journal of Hematology 51:19–25 (1996)

Neonatal Jaundice and Molecular Mutations in Glucose-6-Phosphate Dehydrogenase Deficient Newborn Infants

Ching-Shan Huang, Kun-Long Hung, May-Jen Huang, Yi-Ching Li, Te-Hui Liu, and Tang K. Tang

Departments of Laboratory Medicine (C.-S.H., M.-J.H., Y.-C.L.) and Pediatrics (K.-L.H.), Cathay General Hospital; Institute of Biomedical Sciences, Academia Sinica (T.-H.L., T.K.T.), Taipei, Taiwan

Molecular mutations of the glucose-6-phosphate dehydrogenase (G6PD) gene and clinical manifestations of neonatal jaundice in 112 male and 50 female Chinese neonates with G6PD deficiency were studied. In the 112 males, the nucleotide (nt) 1376 (G→T) mutation was the dominant type (50.0%), followed by nt 1388 (G→A) (16.1%), nt 493 (A→G) (8.0%), nt 1024 (C→T) (6.2%), nt 95 (A→G) (5.4%), nt 392 (G→T) (1.8%), nt 487 (G→A) (1.8%), nt 871 (G→A) (0.9%), and nt 1360 (C→T) (0.9%). The nt 871 variant has not been reported in Taiwan before. The occurrence rates for nt 1376, nt 1388, nt 493, nt 95, and nt 1024 mutations in the 50 females were 44.0%, 18.0%, 12.0%, 6.0%, and 6.0%, respectively. The type of G6PD mutation in 10 male and 7 female neonates has not been identified yet. Although G6PD deficient neonates had higher frequency of phototherapy than G6PD normal neonates in both sexes, a significant difference in the prevalence of hyperbilirubinemia (peak bilirubin ≧15.0 mg/dl) between G6PD deficient and normal neonates was found only in males. Further analysis showed that duration of phototherapy was longer in G6PD deficient male neonates than in the control group, while the outcome of phototherapy was better in subjects with non-nt 1376 mutations than subjects with the nt 1376 mutation. Most (78.3%) of the 23 G6PD deficient neonates who subsequently suffered from neonatal hyperbilirubinemia carried the nt 1376 mutation. The results of this study indicate that the nucleotide substitution at 1376 is the most common and important mutation for G6PD deficiency in Chinese neonates in Taiwan. © 1996 Wiley-Liss, Inc.

Key words: neonatal jaundice, G6PD deficiency, molecular mutants

INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human genetic enzymopathy, affecting over 200 million individuals worldwide, and is closely associated with neonatal jaundice, chronic nonspherocytic hemolytic anemia, favism and food- or drug-induced acute hemolytic anemia [1,2]. Although over 400 G6PD variants based on biochemical studies have been reported, nearly 70 mutations of the G6PD gene have been identified at the DNA level [2–6]. Most of the mutations identified so far are point mutations due to one or two nucleotide substitutions. The eight exceptions are G6PD Vancouver [7] caused by three nucleotide substitutions, G6PD Sunderland [8], G6PD Nara [9], G6PD Stonybrook [4], G6PD Tsukui [5], and G6PD Urayasu [5] caused by deletions in certain parts of necleotides, G6PD Varnsdorf caused by deletion of the ag of the 3′ acceptor splice site of intron 10 [4], and G6PD Georgia caused by nonsense mutation at nucleotide 1284 [4]. We and others have previously shown that at least nine different types of G6PD mutation are responsible for G6PD deficiency in Taiwan [10–14].

The quantitation of G6PD activity for every neonate born at Cathay General Hospital in Taipei has been a routine test since 1980 [15]. This screening test is performed within 3 days of baby's birth. During the past 3 years, molecular diagnosis of G6PD mutations at the DNA level has been established. However, the association between specific DNA mutations and clinical manifestations in neonates has not yet been characterized.

Received for publication March 9, 1995; accepted August 9, 1995.

Address reprint requests to Professor Ching-Shan Huang, Director, Department of Laboratory Medicine, Cathay General Hospital, No. 280, Section 4, Jen-Ai Rd, Taipei, Taiwan, Republic of China.

MATERIALS AND METHODS

Subjects and Quantitation of G6PD

Umbilical cord blood samples were obtained from 4,277 male and 3,771 female neonates born at Cathay General Hospital. The quantitation of G6PD activity in all blood samples was measured by the automated enzyme-coupled method as described [15]. Any subjects with G6PD activity below 4.0 IU/gHb were defined as having G6PD deficiency and their G6PD mutations were then analyzed at the DNA level.

Identification of G6PD Mutations

Nine oligonucleotides with natural or mutagenesis primer sets [10,11,16] used for detection of the nine known G6PD mutations were synthesized on a DNA synthesizer and purified as described [17]. For quick detection of those known mutations, the techniques of direct polymerase chain reaction (PCR) from whole blood [16,18] were modified. One μl of blood containing anticoagulant was added to 10 μl gene-releaser (Bioventures Inc., Murfreesboro, TN), then the thermocycle program was performed on a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) as follows: 1 cycle of 30 sec at 65°C, 30 sec at 8°C, 1 min and 30 sec at 65°C, 3 min at 97°C, 1 min at 8°C, 3 min at 65°C, 1 min at 97°C, 1 min at 65°C, and 5 min at 94°C. After the gene-releaser program, 50 μl of PCR mixture (consisting of 50 ng each primer, 200 μM each dNTP, 10mM Tris-HCl pH 8.8 at 25°C, 1.5mM $MgCl_2$, 50mM KCl, 0.1% Triton X-100, 0.5 U of thermostable DNA polymerase [Dynazyme, Finnzymes Inc., Espoo, Finland]) was added. The PCR amplification was performed on the DNA thermal cycler for 35 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, and final extension at 72°C for 10 min. The PCR product was digested with appropriate restriction enzymes and analyzed on a 3% agarose gel (NuSieve 3:1, FMC Bioproducts, Rockland, ME) containing ethidium bromide [10]. The natural or mutagenesis primers, the restriction enzymes and the digested restriction fragment sizes of the nine known mutations are listed in Table I. The G6PD deficiency subjects who carried an unknown mutation were further analyzed by the PCR-SSCP (single strand conformation polymorphism) technique [19] with modification [20]. DNA sequencing was performed by the dideoxy sequencing method using ^{35}S-dATP and sequenase kit (United States Biochemical Corp., Cleveland, OH) as described [10].

Criteria for Phototherapy and Hyperbilirubinemia

Determination of total serum bilirubin was performed on each neonate every day during hospitalization with a Bil Analyzer A 7001 (Nakamura Inc., Tokyo). The criteria for phototherapy were as suggested by King and Jung [21]. Phototherapy was ended if the serum bilirubin concentration declined below half of the level for exchange transfusion suggested by Gartner and Whitington [22]. The definition of neonatal jaundice was that a newborn infant needed phototherapy, while neonatal hyperbilirubinemia was defined by a peak bilirubin level $\geqq$15.0 mg/dl in serum.

Risk Factors for Jaundice

Except for G6PD deficiency, the risk factors for jaundice include delivery by Caesarean section, premature birth, low birth weight, hypoxia/asphyxia, ABO incompatibility, having a hepatitis B surface antigen (HBsAg) carrier mother [23] and sepsis.

Statistical Analysis

For the 4,277 male and 3,771 female neonates screened for G6PD activity, the frequency of phototherapy and the prevalence of hyperbilirubinemia in G6PD deficient neonates were compared with those in G6PD normal neonates, male and female separately. If the difference in the frequency for phototherapy and the prevalence of hyperbilirubinemia were both statistically significant, a further study was performed to determine whether the dominant mutation type was also the chief mutation associated with clinical manifestations. In the further study, the clinical data on neonatal jaundice for the dominant type of G6PD mutations were compared with data for other mutations, and 300 G6PD normal neonates were selected as the control group. Except for G6PD deficiency, the risk factors for neonatal jaundice in the control subjects were matched to those in G6PD deficient neonates. Chi-square test, Yates' correction, or Fisher's exact probability tests as appropriate, were used to compare the frequency of phototherapy and the prevalence of hyperbilirubinemia in groups. Student's t-test was used to compare the duration of phototherapy and peak bilirubin value between each of the two groups for the dominant mutation, other mutations, and control groups. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Among the 4,277 male and 3,771 female neonates screened for G6PD activity, 112 males and 50 females were found to be G6PD deficient. By the quick method for the detection of known G6PD mutations, the mutation types in 101 subjects out of the 112 males were identified as those listed in Table II. The blood samples of the other 11 G6PD deficient males were then analyzed by the modified SSCP method, and one sample was found to be a new mutation—a nucleotide substitution at position 871. Figure 1 shows the nucleotide sequence of the PCR product for the nt 871 mutation. The mutation type of the remaining 10 subjects has not yet been identified. Thus, the frequencies of various mutations in the 112

TABLE I. Natural or Mutagenesis Primers, Restriction Enzymes, and the Results for G6PD Mutations

Position (cDNA)	Primers	Sequence	Restriction enzyme	Results (bp[b])
95	95F :	5'CTCTAGAAAGGGGCTAACTTCTCA3'	Mlu I	N[c] 198
A→G	95R :	5'GATGCACCCATGATGATGAATACG3'[a]		M[d] 174+24
392	392F :	5'GGACTCAAAGAGAGGGGCTG3'	BstE II	N 188+15
G→T	392R :	5'GAAGAGGCGGTTGGCCGGTGAC3'		M 203
487	487F :	5'GCGTCTGAATGATGCAGCTCTGAT3'	Hind III	N 104
G→A	487R :	5'CTCCACGATGATGCGGTTCAAGC3'		M 82+22
493	493F :	The same as for nt 487	Ava II	N 120+11
A→G	493R :	5'CTCTGCAGGTCCCTCCCGAAGGGC3'		M 87+33+11
592	592F :	5'GAGGAGGTTCTGGCCTCTACTC3'	Pst I	N 157+83
C→T	592R :	5'TTGCCCAGGTAGTGGTCGCTGC3'		M 157+63+20
1024	1024F :	5'GTCAAGGTGTTGAAATGCATC3'	Mbo II	N 187
C→T	1024R :	5'CATCCCACCTCTCATTCTCC3'		M 150+37
1360	1360F :	5'ACGTGAAGCTCCCTGACGC3'	Hha I	N 142+45+27
C→T	1360R :	5'GTGAAAATACGCCAGGCCTTA3'		M 187+27
1376	1376F :	The same as for nt 1360	Afl II	N 214
G→T	1376R :	The same as for nt 1360		M 194+20
1388	1388F :	The same as for nt 1360	Nde I	N 227
G→A	1388R :	5'GTGCAGCAGTGGGGTGAACATA3'		M 206+21

[a]'_': mutagenesis site.
[b]bp: base pair (size of PCR product).
[c]N: normal digestion result.
[d]M: mutant digestion result.

male G6PD deficient neonates were as follows: 50% for nt 1376, 16.1% for nt 1388, 8.0% for nt 493, 6.2% for nt 1024, 5.4% for nt 95, 1.8% for nt 392, 1.8% for nt 487, 0.9% for nt 871, 0.9% for nt 1360, and 8.9% for unknown. The G6PD activity in G6PD deficient male neonates was very low, ranging from 0 to 2.5 IU/gHb. The mutation type of 43 subjects out of 50 G6PD deficient females was analyzed by the quick DNA diagnosis method, while in the other seven samples (14.0%) the mutation type has not been identified yet. As the data in Table II show, among the 50 females, the nt 1376 mutation was also the dominant type (44.0%), followed by nt 1388 (18.0%), nt 493 (12.0%), nt 95 (6.0%), and nt 1024 (6.0%). The G6PD activity in the 50 G6PD deficient female neonates was moderately low, ranging from 0 to 4.0 IU/gHb. The frequency of phototherapy (36.6%) and the prevalence of hyperbilirubinemia (20.5%) in G6PD deficient male neonates were significantly higher than those in G6PD normal male neonates (13.8% and 7.2%) ($P < 0.001$) (Table III). In female neonates, the frequency of phototherapy was also higher in G6PD deficient subjects than in G6PD normal subjects (24.0% vs. 10.8%, $P = 0.005$), while the prevalence of hyperbilirubinemia was no different between G6PD deficient and G6PD normal groups (8.0% vs. 5.9%, $P = 0.564$). Table IV shows a comparative analysis of the risk factors for jaundice in male newborns that carried either the nt 1376 or non-nt 1376 mutations. However among the 27 subjects analyzed, no particular risk factor was associated with either nt 1376 or non-nt 1376 carriers. The data in Table V indicate that the duration of phototherapy in nt 1376 (3.5 ± 1.6 days) and other nt mutations (3.9 ± 2.0 days) was significantly longer than that (2.3 ± 1.3 days) in the control group ($P < 0.001$). The results also show that the mean value of peak bilirubin and the prevalence of hyperbilirubinemia in the 56 subjects with nt 1376 mutation who received phototherapy were statistically higher than those in the other 56 subjects with non-nt 1376 mutation and the 300 controls ($P < 0.001$), while no significant difference was observed between the subjects with non-nt 1376 mutation and the normal controls ($P \geqq 0.09$). We also found that the nt 1376 mutation accounted for 78.3% (18/23) of G6PD deficient males suffering from neonatal hyperbilirubinemia. However, the number of subjects who needed blood exchange treatment was no different among subjects with the nt 1376 mutation, other nt mutations, and the control group ($P \geqq 0.5$). Table VI shows that in the subjects who carried the nt 1376 mutation, neither phototherapy ($P = 0.462$) nor hyperbilirubinemia ($P = 0.191$) were associated with combined risk factors.

DISCUSSION

We [10,14] and others [12,13] have previously shown that at least nine different types of mutation are responsible for G6PD deficiency in Taiwan. Our results show that the occurrence rates of G6PD mutations in neonates are similar to that reported by Lo et al. [13], except that nt 592 is absent while nt 871 represents a new finding in

TABLE II. G6PD Mutations in Male and Female Newborn Infants

Variant	Nucleotide substitution	Male newborn infants N	%	G6PD activity (IU/gHb) X ± SD	Range	Female newborn infants[c] N	%	G6PD activity (IU/gHb) X ± SD	Range
Taiwan-Hakka, Gifu-like	1376	56	50.0	0.1 ± 0.2	0 ~ 0.8	22	44.0	2.3 ± 1.0	0.4 ~ 3.8
Kaiping, Anant, Dhon, Petrich, Sapporo	1388	18	16.1	0.4 ± 0.4	0 ~ 1.2	9	18.0	3.0 ± 0.8	1.2 ~ 4.0
G6PD-Taipei	493	9	8.0	0.5 ± 0.7	0 ~ 2.1	6	12.0	2.8 ± 0.7	2.0 ~ 3.6
Chinese-5	1024	7	6.2	1.2 ± 0.9	0 ~ 2.5	3	6.0	2.9 ± 0.9	2.0 ~ 3.7
Gaohe	95	6	5.4	0.1 ± 0.1	0 ~ 0.2	3	6.0	2.8 ± 0.9	1.9 ~ 3.7
Chinese-4	392	2	1.8	(1.6, 0.2)					
Mahidol	487	2	1.8	(0.0, 0.6)					
Viangchan, Jammu	871[a]	1	0.9	(0)					
Union, Maewo	1360[b]	1	0.9	(0)					
	Unknown	10	8.9	0.6 ± 0.5	0 ~ 1.3	7	14.0	2.3 ± 1.3	0 ~ 3.8
	Total	112	100			50	100		

[a]The nt 871 mutation was identified by the modified SSCP method [20], while the other mutations were detected by the quick DNA diagnosis method [16,18].
[b]Mother of the baby is a Philippine.
[c]All the female subjects of the known G6PD mutations were heterozygous mutations.

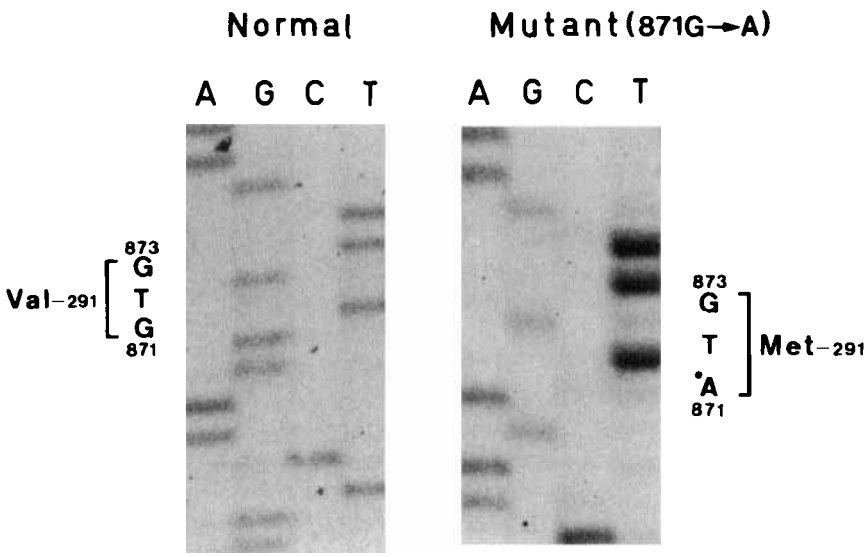

Fig. 1. Sequence analysis of the PCR-amplified fragments of normal and affected individuals. The amplified fragments were sequenced as described in Materials and Methods.

TABLE III. Frequency of Phototherapy and Prevalence of Hyperbilirubinemia in Newborn Infants

	Male newborn infants Total N	Phototherapy N (%)	Hyperbilirubinemia N (%)	Female newborn infants Total N	Phototherapy N (%)	Hyperbilirubinemia N (%)
G6PD deficiency	112	41 (36.6)	23 (20.5)	50	12 (24.0)	4 (8.0)
G6PD normal	4165	575 (13.8)	300 (7.2)	3721	402 (10.8)	190 (5.1)
P-value		<0.001*	<0.001*		0.005*	0.564**

*Chi-square test.
**Yates' correction test.

TABLE IV. Risk Factors for Neonatal Jaundice in Male Newborn Infants With G6PD Deficiency

	Nucleotide substitution at 1376 (N = 56)	Other mutations (N = 56)
Caesarean section	4	2
Premature birth	3	6
Low birth weight	1[a]	0
Hypoxia/asphyxia	0	1
ABO incompatibility	5	6
HBsAg carrier mother	6	7
Multiple factors	8	5
Total number with risk factors	27	27

[a]Birth weight was 2400 g.

TABLE VI. Comparison of Numbers of Subjects With Nucleotide Substitution at 1376, With and Without Risk Factors

	N	N phototherapy	N hyperbilirubinemia
With risk factors	27	13	11
Without risk factors	29	11	7
P-value		0.462[a]	0.191[a]

[a]*P*-values are given for chi-square test.

Taiwan. The difference could be because the nt 592 mutation seems to affect Ami people (native Taiwan aborigines) [14], whose newborn infants are not included in our studies. The nt 871 mutation, originally described by Beutler et al. [24] is common in Southeast Asia [25,26]. However, we reported here for the first time that the same mutation was also found in Chinese neonates in Taiwan. We can conclude that there are at least 10 mutation types of G6PD deficiency in Taiwan. Among the 10 mutation types, nt 1376 predominates. Out of the 112 male and 50 female G6PD deficient subjects we studied, 10 males and 7 females did not show any of the known mutations. These results suggest that more unknown G6PD mutations may be present in the population of Taiwan.

It has been found that G6PD deficiency is definitely associated with neonatal jaundice in many areas [27]. In our study, we found that in both sexes the frequency of phototherapy for G6PD deficient neonates is significantly higher than that for G6PD normal neonates (Table III). This might be because excessive production of bilirubin and impaired hepatic clearance of serum bilirubin occur more frequently in G6PD deficient neonates than in G6PD normal neonates. However, a significant difference in the prevalence of hyperbilirubinemia between G6PD deficient and G6PD normal neonates was found only in males, not in females (Table III). The tendency to develop neonatal hyperbilirubinemia in G6PD deficient female neonates is known to be related to the degree of G6PD mosaicism [28,29]. As the G6PD activity of the 50 G6PD deficient female neonates was moderately low and 43 of them were confirmed to be heterozygous by molecular techniques, it is not surprising that a lower prevalence of neonatal hyperbilirubinemia was found among those female subjects.

As nt 1376 accounted for 50% of G6PD deficient male neonates in this study, the clinical manifestations of the subjects with nt 1376 were compared with those of the other 50% of subjects who carried other nt mutations. The frequency of each risk factor for jaundice was almost evenly distributed between the subjects with nt 1376 and those with other nt mutations (Table IV). The mean value of the duration for phototherapy in G6PD deficient male neonates was one day longer than that in the risk factor-matched controls (Table V). The significant difference in the prevalence of hyperbilirubinemia between male neonates with nt 1376 and non-nt 1376 mutations reveals the outcome of phototherapy for non-nt 1376 mutations is better than that for nt 1376 mutation. Our results also indicate that the nt 1376 mutation accounted for 78.3%

TABLE V. Clinical Data in Newborn Infants With G6PD Deficiency and Risk-Factor-Matched Controls

		Total N	Phototherapy N (%)	Phototherapy Duration of treatment (days, X ± SD)	Peak bilirubin of infants with phototherapy X ± SD mg/dl	Peak bilirubin of infants with phototherapy N (%) ≧15.0 mg/dl	Peak bilirubin of infants with phototherapy N (%) ≧20.0 mg/dl
Nucleotide substitution at 1376		56	24 (42.8)	3.5 ± 1.6	15.8 ± 1.9	18 (32.1)	1 (1.8)
Other mutations		56	17 (30.4)	3.9 ± 2.0	14.5 ± 2.0	5 (8.9)	0 (0)
Controls		300	43 (14.3)	2.3 ± 1.3	14.9 ± 1.7	21 (7.0)	3 (1.0)
P-value	Nt 1376 vs. controls		<0.001*	<0.001**	<0.001**	<0.001*	0.866***
	Other mutations vs. controls		0.005	<0.001	0.09	0.824	0.967
	Nt 1376 vs. other mutations		0.177	0.778	<0.001	<0.001	0.5†

*Chi-square test.
**Student's t test.
***Yates' correction test.
†Fisher's exact test.

of the subjects with hyperbilirubinemia in G6PD deficient male neonates. Lo et al. [13] reported that in South Taiwan, the nt 1376 mutation was the most common mutation (64.7%) found in G6PD deficient male infants who required blood exchange treatment. Taken together, those results indicate that nt 1376 is the most important mutation for G6PD deficient Chinese neonates in Taiwan. Since there is no evidence to support the concept that neonatal hyperbilirubinemia is attributable to the risk factors described in Table VI, the real causes of hyperbilirubinemia in neonatal subjects who carry the nt 1376 mutation are worthy of further study.

Although the nt 1376 mutation is a high risk mutation for neonatal hyperbilirubinemia, only one subject with this mutation subsequently needed blood exchange treatment. During hospitalization, this male neonate received phototherapy and the peak serum bilirubin level was 13.5 mg/dl. His parents asked for this discharge from hospital on the eighth day after his birth, despite our advice. On the ninth day after birth, his parents gave him a Chinese herbal drug named green-leaf-juice. One day later, this baby was brought back to our hospital because of the deep yellow color of his skin. The serum bilirubin level rose up to 22.1 mg/dl and then 50 ml of blood from this baby was exchanged. The Chinese herbal drug was probably a strong oxidant that induced the development of severe neonatal hyperbilirubinemia. Except for this special case, the peak bilirubin level of other male neonates with G6PD deficiency was not higher than 20.0 mg/dl. From these experiences, we conclude that in time, screening and health education as performed at Cathay General Hospital may lead to the decline of severe neonatal hyperbilirubinemia caused by G6PD deficiency. This conclusion is similar to a report described by Singh [30]. Recently, Meloni et al. [31] have reported that the cases of favism declined remarkably after performing neonatal G6PD screening tests and health education. Therefore, the program of G6PD screening is a cost-effective medical service in areas where the incidence of G6PD deficiency is high.

ACKNOWLEDGMENTS

This study was supported by a research grant from Cathay General Hospital, Taipei, Taiwan.

REFERENCES

1. Beutler E: The genetics of glucose-6-phosphate dehydrogenase deficiency. Semin Hematol 27:137–164, 1990.
2. Beutler E: G6PD deficiency. Blood 84:3613–3636, 1994.
3. Vulliamy T, Beutler E, Luzzatto L: Variants of glucose-6-phosphate dehydrogenase are due to missense mutations spread throughout the coding region of the gene. Hum Mutat 2:159–167, 1993.
4. Xu W, Westwood B, Bartsocas CS, et al: G6PD mutations and haplotypes in various ethnic groups. Blood 85:257–263, 1995.
5. Hirono A, Fujii H, Miwa S: Identification of two novel deletion mutations in glucose-6-phosphate dehydrogenase gene causing hemolytic anemia. Blood 85:1118–1121, 1995.
6. Mason PJ, Sonati MF, MacDonald D, et al: New glucose-6-phosphate dehydrogenase mutations associated with chronic anemia. Blood 85: 1377–1380, 1995.
7. Maeda M, Constantoulakis P, Chen C-S, Stamatoyannopoulos G, Yoshida A: Molecular abnormalities of a human glucose-6-phosphate dehydrogenase variant associated with undetectable enzyme activity and immunologically cross-reacting material. Am J Hum Genet 51:386–395, 1992.
8. MacDonald D, Town M, Mason P, Vulliamy T, Luzzatto L: Deficiency in red blood cells. Nature 350:115, 1991.
9. Hirono A, Fujii H, Shima M, Miwa S: G6PD Nara: a new class I glucose-6-phosphate dehydrogenase variant with an eight amino acid deletion. Blood 82:3250–3252, 1993.
10. Tang TK, Huang CS, Huang MJ, Yeh CH, Tang C-JC: Diverse point mutations result in glucose-6-phosphate dehydrogenase (G6PD) polymorphism in Taiwan. Blood 79:2135–2140, 1992.
11. Chang JG, Chiou SS, Perng LI, Chen TC, Liu TC, Lee LS, Chen PH, Tang TK: Molecular characterization of G6PD deficiency by natural and amplification created restriction sites: five mutations account for most G6PD deficiency cases in Taiwan. Blood 80:1079–1082, 1992.
12. Chiu DTY, Zuo L, Chao L, et al: Molecular characterization of glucose-6-phosphate dehydrogenase (G6PD) deficiency in patients of Chinese descent and identification of new base substitutions in the human G6PD gene. Blood 81:2150–2154, 1993.
13. Lo YS, Lu CC, Chiou SS, Chen BH, Chang TT, Chang JG: Molecular characterization of glucose-6-phosphate dehydrogenase deficiency in Chinese infants with or without severe neonatal hyperbilirubinemia. Br J Haematol 86:858–862, 1993.
14. Tang TK, Yeh CH, Huang CS, Huang MJ: Expression and biochemical characterization of human glucose-6-phosphate dehydrogenase in *Escherichia coli:* a system to analyze normal and mutant enzymes. Blood 83:1436–1441, 1994.
15. Huang CS, Chen TH, Wei C, Chein TY, Jang JF: The clinical application of glucose-6-phosphate dehydrogenase quantitative test. J Formosan Med Assoc 81:938–944, 1982.
16. Tang TK, Yeh CH, Huang CS, Huang MJ, Tang C-JC, Chang JG: Molecular diagnosis of glucose-6-phosphate dehydrogenase (G6PD) mutations. J Genet Mol Biol 3:47–59, 1992.
17. Tang TK, Qin Z, Leto T, Marchesi VT, Benz EJ Jr: Heterogeneity of mRNA and protein products arising from the protein 4.1 gene in erythroid and nonerythroid tissues. J Cell Biol 110:617–624, 1990.
18. Mercier B, Gaucher C, Feugeas O, Mazurier C: Direct PCR from whole blood, without DNA extraction. Nucleic Acids Res 18:5908, 1990.
19. Hirono A, Miwa S, Fujii H, Ishida F, Yamada K, Kubota K: Molecular study of eight Japanese cases of glucose-6-phosphate dehydrogenase deficiency by nonradioisotopic single-strand conformation polymorphism analysis. Blood 83:3363–3368, 1994.
20. Liu TC: Molecular analysis of G6PD mutation and polymorphism in Taiwan Chinese and aborigines [thesis for Master of Science]. National Taiwan University, 1994.
21. King JD, Jung AC: Phototherapy. In Nelson NM (ed): Current therapy in neonatal-perinatal medicine-2. Philadelphia: BC Decker, 1990, pp 461–464.
22. Gartner CM, Whitington PF: Disorders of bilirubin metabolism. In Nathan DG (ed): Hematology of infancy and childhood. Philadelphia: WB Saunders, 1987, pp 74–103.
23. Yu MW, Hsiao KJ, Wuu KD, Chen CJ: Association between glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice. Int J Epidem 21:947–952, 1992.
24. Beutler E, Westwood B, Kuhl W: Definition of the mutations of G6PD Wayne, G6PD Viangchan, G6PD Jammu and G6PD "Le Jeune." Acta Haematol (Basel) 86:179–182, 1991.

25. Beutler E, Westwood B, Kuhl W, Hsia YE: Glucose-6-phosphate dehydrogenase variants in Hawaii. Hum Hered 42:327–329, 1992.
26. Hsia YE, Miyakawa F, Baltazar J, Ching NSP, Yuen J, Westwood B, Beutler E: Frequency of glucose-6-phosphate dehydrogenase (G6PD) mutations in Chinese, Filipinos, and Laotians from Hawaii. Hum Genet 92:470–476, 1993.
27. Piomelli S: G6PD-related neonatal jaundice. In Yoshida A, Beutler E (eds): Glucose-6-phosphate dehydrogenase. Orlando, FL: Academic Press, 1986, pp 95–108.
28. Sanna G, Frau F, De Virigiliis S, et al: Glucose-6-phosphate dehydrogenase red blood cell phenotype in Gd Mediterranean heterozygous females and hemizygous males at birth. Pediatr Res 15:1443–1446, 1981.
29. Meloni T, Forteleoni G, Dore A, et al: Neonatal hyperbilirubinemia in heterozygous glucose-6-phosphate dehydrogenase deficient females. Br J Haematol 53:241–246, 1983.
30. Singh H: Glucose-6-phosphate dehydrogenase deficiency: a preventable cause of mental retardation. Br Med J 292:397, 1986.
31. Meloni T, Forteleoni G, Meloni GF: Marked decline of favism after neonatal glucose-6-phosphate dehydrogenase screening and health education: the Northern Sardinian experience. Acta Haematol (Basel) 87:29–31, 1992.